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SYSTEMATIC REVIEW

Immunohistochemical predictive markers of response to conservative treatment of endometrial hyperplasia and early endometrial cancer: A systematic review

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Abstract

Introduction: Progestogens are widely used for the conservative treatment of endometrial hyperplasia and early endometrial cancer. Nevertheless, they do not achieve the regression in all cases. Although several immunohistochemical markers have been assessed to predict the response to treatment, their usefulness is still unclear. We aimed to analyze the usefulness of each immunohistochemical marker studied in predicting the response to progestogens in endometrial hyperplasia and early endometrial cancer.

Material and methods: Electronic databases were searched for relevant articles from January 2000 to June 2018. All studies assessing the association of immunohistochemical markers with the outcome of the progestogen-based therapy in endometrial hyperplasia and early endometrial cancer were included. The expression of immunohistochemical markers in pretreatment phase and changes of expression during the follow-up were evaluated in relation to response to therapy and relapse.

Results: Twenty-seven studies with 1360 women were included in the systematic review; 43 immunohistochemical markers were assessed. The most studied predictive markers in the pretreatment phase were progesterone and estrogen receptors, although with conflicting results; their isoforms, and in particular progesterone receptor B, appeared more promising. Further studies are needed to confirm the usefulness of mismatch repair proteins, Dusp6, GRP78 and PTEN combined with other molecules such as phospho-AKT or phospho-mTOR. In the follow-up phase, Nrf2 and survivin showed the stronger evidence; a role may also be played by Bcl2 and Ki67. Further studies are necessary for Fas, NCoR, AKR1C1, HE4, PAX2 and SPAG9.

Conclusions: Several immunohistochemical markers might be helpful in predicting the response to conservative treatment of endometrial hyperplasia and early endometrial cancer on pretreatment and follow-up specimens. Further studies are needed

Abbreviations: AKR1c1, aldo-keto reductase family 1 member c1; Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; Dusp6, dual-specificity phosphatase 6; EEC, early endometrial cancer; EH, endometrial hyperplasia; ER, estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; Fas, fas cell surface death receptor; FOXO1, forkhead box protein O1; GRP78, glucose-regulated protein-78; HE4, Human epididymis protein 4; Ki67, antigen Ki67; LNG-IUS, levonorgestrel-releasing intrauterine system; MMR, mismatch repair proteins; NCoR, nuclear receptor co-repressor; Nrf2, nuclear factor erythroid 2-related factor 2; PAX2, paired box gene 2; phospho-mTOR, phospho-mammalian Target of rapamycin; PRA, progesterone receptor A; PRB, progesterone receptor B; PTEN, phosphatase and tensin homolog; SMRT, silencing mediator for retinoid and thyroid-hormone receptors; SPAG9, sperm-associated antigen 9; SRC1, steroid receptor coactivator-1.

to confirm their usefulness and possibly integrate them in a predictive immunohistochemical panel.

KEYWORDS

endometrioid adenocarcinoma, endometrial cancer, endometrial hyperplasia, endometrial intraepithelial neoplasia, immunohistochemical marker, progestin, Progestogens

1 | INTRODUCTION

Endometrial hyperplasia (EH) is an irregular proliferation of endometrial glands with increased gland to stroma ratio when compared with proliferative endometrium.[1] EH is the precursor of endometrioid endometrial adenocarcinoma, the most common histotype of the most prevalent gynecological cancer in the developed world.[2-4]

Endometrial hyperplasia may be a polyclonal proliferative lesion or a monoclonal precancerous lesion, differentiated on the basis of cytologic atypia by 2014 World Health Organization classification.[1,5,6]

Although the gold standard treatment for precancerous EH and endometrial cancer is hysterectomy, many patients need conservative treatment to preserve fertility or to avoid surgery at high risk. Conservative treatment consists of progestins and follow-up biopsies every 3-6 months.[6,7] Eligibility criteria for conservative treatment may also be extended to early endometrial cancer (EEC), ie, endometrial cancer with endometrioid type, tumor grade 1, absence of lymphovascular space, myometrial or cervical invasion and absence of extrauterine metastases.[8] Although several progestogens have been used for conservative treatment (megestrol acetate, medroxyprogesterone acetate, norethindrone acetate and levonorgestrel), levonorgestrel-releasing intrauterine system (LNG-IUS) seems to be the most effective one.[6,9] However, a considerable percentage of patients does not respond to conservative treatment, or show relapse after a remission, with the risk of progression to invasive disease.[10] For this reason, in the last years there has been a growing interest in the study of clinical, imaging, histological and molecular factors that may influence the outcome of therapy.[11-13] Immunohistochemistry—which is the most used tool in the assessment of tissue markers for the diagnosis, prognosis and therapy of a great number of diseases[14] has played a major role in this field. Although a great number of immunohistochemical markers have been assessed, their usefulness is still unclear.

Thus, our aim was to systematically review the available literature regarding the usefulness of immunohistochemical markers in predicting the outcome of conservative therapy in EH and EEC.

Key message

The most useful markers of response to conservative treatment of endometrial hyperplasia and cancer may be progesterone receptor and estrogen receptor isoforms in the pretreatment phase and Nrf2, survivin, Bcl2 and Ki67 on follow up. Further studies are needed for several other promising markers.

2 | MATERIAL AND METHODS

This study was performed according to a protocol recommended for systematic review. The review protocol was designed a priori, defining methods for collecting, extracting and analyzing data. All review stages were conducted independently by two reviewers (A.R., A.T.). The two authors independently assessed electronic search, eligibility of the studies, inclusion criteria, risk of bias, data extraction and data analysis. Disagreements were resolved by discussion with a third reviewer (G.S.).

The study was reported according to the Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement.

The research was conducted using MEDLINE, Embase, Web of Sciences, Scopus, ClinicalTrial.gov, OVID and Cochrane Library as electronic databases. The studies were identified with the use of a combination of the following text words from January 2000 to June 2018: endometrial hyperplasia; endometrial cancer; endometrioid adenocarcinoma; endometrial intraepithelial neoplasia; EIN; therapy; treatment; fertility sparing; conservative; medroxyprogesterone; MPA; mirena; LNG; levonorgestrel; progesterone; progestogen; progestin; response; resistance; persistence; relapse; recurrence; progression; outcome; immunohistochemistry; immunohistochemical. Review of articles also included the abstracts of all references retrieved from the search.

We included in our systematic review all randomized and non-randomized studies that satisfied the following inclusion criteria:

- study population constituted of women diagnosed with EH or EEC and conservatively treated with progestogens;
- assessment of the expression of one or more immunohistochemical markers on endometrial biopsies or curettages in pretreatment and/or follow-up phase;
- assessment of the association between expression of immunohistochemical markers and outcome of therapy.

The risk of bias was assessed via the Methodological Index for Non-Randomized Studies (MINORS). Seven domains related to risk of bias were assessed in each study: (1) aim (ie, clearly stated aim), (2) rate (ie,

inclusion of consecutive patients and response rate), (3) data (ie, prospective collection of data), (4) bias (ie, unbiased assessment of study endpoints), (5) time (ie, follow-up time appropriate), (6) loss (ie, loss to follow up), (7) size (ie, calculation of the study size). Review authors' judgments were categorized as "low risk" , "high risk" or "unclear risk of bias" .

Data were extracted from the included studies without modifications. The main data extracted for our systematic review were:

- the immunohistochemical expression of the predictive markers, evaluated as "absent", "low" or "high"; and
- the outcome of conservative treatment, dichotomized into "good response" vs "poor response" and/or "relapse" vs "no relapse".

The association between marker expression and therapy outcome was assessed separately in the pretreatment phase and follow-up phase.

Secondary data was extracted regarding patient age and body mass index, pathological diagnosis, progestogen type and administration route, and treatment duration.

3 | RESULTS

Twenty-seven studies,[15-41] with a total of 1360 patients and 43 immunohistochemical markers assessed, were included in this systematic review (Figure 1): 20 studies were retrospective and seven were prospective.

Results of bias assessment are shown in Figure 2.

The age of the patients ranged between 19 and 79 years. The body mass index ranged between 17 and 72 kg/m^2. The sample size ranged from 7 to 174 and included 629 EH without atypia (in 11 studies), 422 atypical EH (in 20), 140 unspecified EH (in 3), and 204 EEC (in 14).

The most used progestogen was medroxyprogesterone acetate (in 23 studies), followed by LNG-IUS (in 14), megestrol acetate (in 8), norethindrone acetate (in 5) and progesterone (in 2). In 6 studies, multi-progestogen treatments were administered. The duration of treatment ranged from 1 week to 90 months. Details about samples and treatment are shown in Table 1.

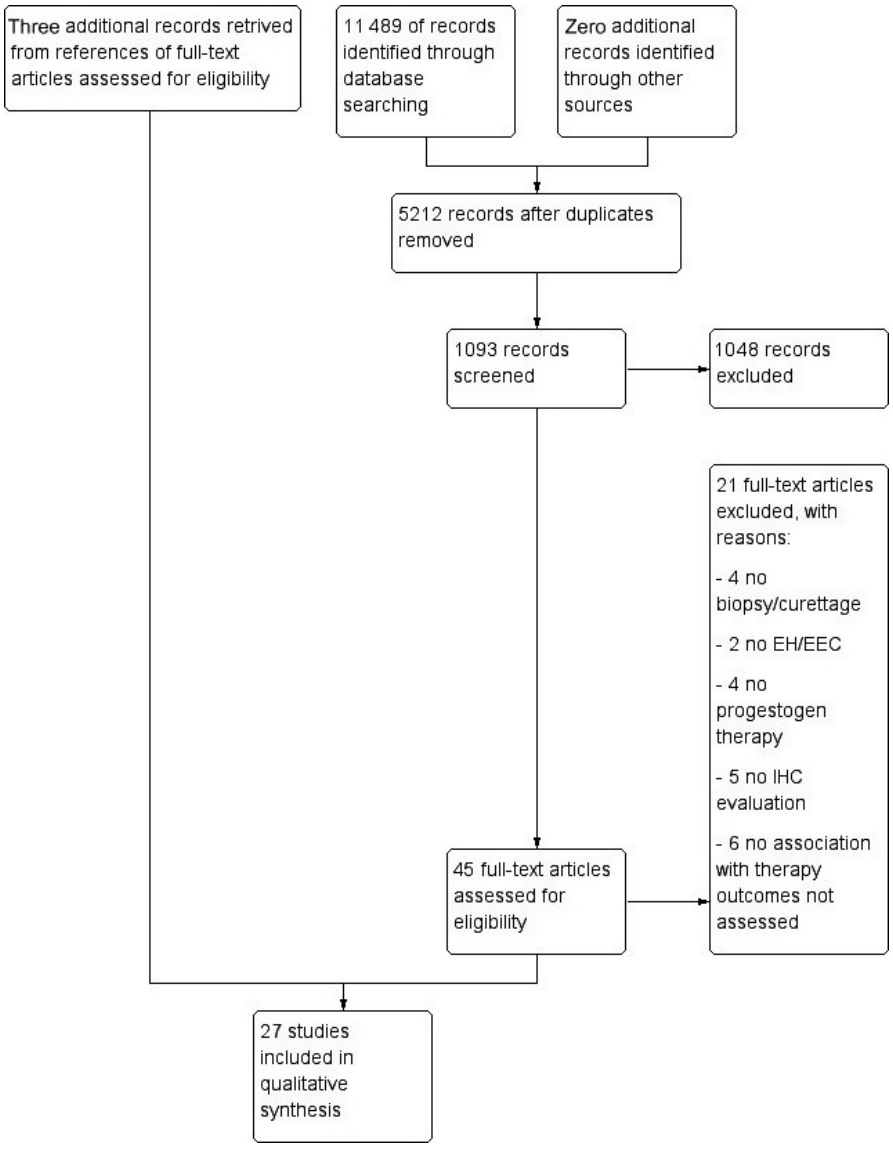

FIGURE 1 Flow diagram of studies identified in the systematic review (Prisma template [Preferred Reporting Item for Systematic Reviews and Meta-analyses])

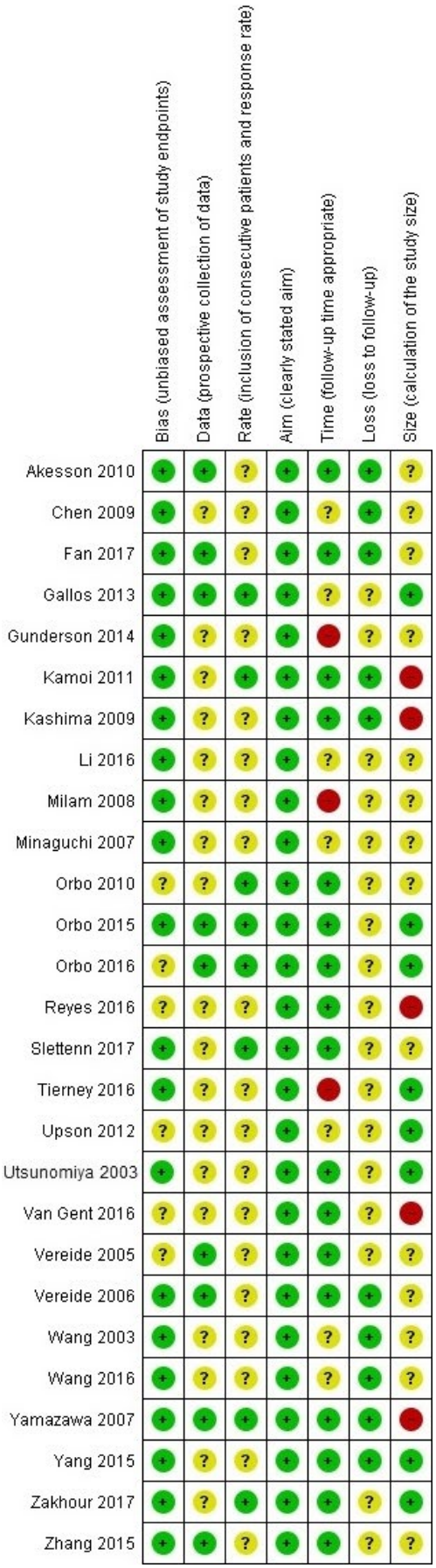

FIGURE 2 Assessment of risk of bias. Summary of risk of bias for each study; Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias [Color figure can be viewed at wileyonlinelibrary.com]

The histological definition of "good response" variably included: complete regression of disease,[16,20-22,28,29,33,35,36,40] progestogen-related effects,[17,18,24,25,30,37-40] atypia disappearance,[15,23,31,41] cancer disappearance,[26] complexity disappearance,[27] no progression,[34] avoided hysterectomy.[19] Four studies considered the outcome "relapse" vs "no relapse".[28,31,37,40]

Nineteen studies assessed pretreatment expression of the markers, and 19 studies assessed post-treatment expression and/or changes of expression during follow up.

Details about outcomes considered, markers assessed with full names and associations found are reported in Table 2.

With specific regard to pretreatment assessment, 19 studies assessed a total of 31 markers on pretreatment biopsy. They searched for predictive markers of response to therapy (in 18 studies) and/or relapse of disease (in three studies).

Progesterone receptor (PR) or its isoforms (PRA and PRB), and estrogen receptor (ER) or its isoforms (ERα and ERβ) were assessed in 12 studies.

A significant association with good response was found for a high expression of PR,[15,20,28] PRA,[25] PRB,[25,27] ER[28] and ERα.[25] Nevertheless, other studies did not find significant associations for PR,[23,26,29,31,34] PRA,[18,27] PRB,[18] ER,[15,23,26,29,31,34] ERα[18,20] or ERβ.[18] A significant association with relapse was shown for low stromal PRA and high glandular PRB expression,[40] but not for ER, PR,[28] ERα, ERβ.[40]

Phosphatase and tensin homolog (PTEN) was assessed in 6 studies. Loss of PTEN predicted poor response only if combined with low phospho-AKT expression,[19] but it was never significant alone[20,21,25,27,35] and did not predict relapse.[40]

Regarding mismatch repair proteins (MMR), an abnormal MMR pattern (including MLH1, MSH2, MSH6, PMS2) strongly predicted poor response.[41] MLH1 alone was not significant.[28]

Forest plots reporting relative risk of poor response for PR, ER, PTEN and MMR are shown in Figure 3.

Furthermore, high expression of dual-specific phosphatase 6 (Dusp6) was predictive of good response,[32] whereas high expression of glucose-regulated protein 78 (GRP78) was predictive of poor response.[33]

High expression of 17 β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) was found predictive of good response.[15]

No association with the outcome was found for 17β-HSD1,[15] B-cell lymphoma 2 (Bcl2),[17,27,28,40] Bcl-2-associated X protein (BAX),[17,40] androgen receptor,[18] tumor protein p53,[19] insulin-like growth factor 1 receptor (IGF1R),[20] antigen Ki67 (Ki67),[15,20,23,26,31] p27,[21] phospho-mammalian Target of rapamycin (phospho-mTOR),[21] steroid receptor coactivator-1 (SRC1),[23] p300/CREB-binding protein (p300/CBP),[23] nuclear receptor co-repressor (NCoR),[23] silencing mediator for retinoid and thyroid-hormone receptors (SMRT),[23] aromatase,[25]

TABLE 1 Characteristics of the included studies (design, sample, treatment)

Year	Article	Study design	Sample size	Patients' features		Histology				Progestogen administered								Treatment duration (mo)
				Age	BMI	HWA	AH	EH	EEC	MGA	NETA	MPA	PG	OR	INJ	LNG	MIX	
2003	Utsunomiya[15]	Retrospective	16	26-38	ns	—	—	—	—	—	—	—	—	—	—	—	—	6-12
	Wang[16]	Retrospective	26	23-51	ns	17	9	—	—	4	3	18	1	—	—	—	—	2-12
2005	Vereide[17]	Prospective	68	28-60	ns	—	—	68	—	—	—	36[b]	—	—	—	32[b]	—	3
2006	Vereide[18]	Prospective	50 (29)[a]	30-70	18-43	37	13	—	—	—	—	29	—	—	—	21		3
2007	Minaguchi[19]	Retrospective	31	19-60	ns	—	12	—	19	—	—	31	—	—	—	—	—	2-18
	Yamazawa[20]	Prospective	9	28-40	ns	—	—	—	9	—	—	9	—	—	—	—	—	6-9
2008	Milam[21]	Retrospective	38	20-79	ns	13	25	—	—	18	5	16[c]	—	—	—	—	—	1-12
2009	Chen[22]	Retrospective	23	23-50	ns	14	9	—	—	4	3	15	1	—	—	—	—	2-12
	Kashima[23]	Retrospective	15	20-49	ns	2	8	—	5	—	—	15	—	—	—	—	—	4-6
2010	Orbo[24]	Retrospective	41	32-55	20-43	39	2	—	—	—	—	16	—	—	—	25	—	6
	Akesson[25]	Prospective	34	36-77	21-49	29	5	—	—	—	—	—	—	—	—	34	—	26
2011	Kamoi[26]	Retrospective	7	20-36	23-49	—	—	—	7	—	—	7	—	—	—	—	—	3-8
2012	Upson[27]	Retrospective	114	<39 to >70	<25 to >30	73	41	—	—	46	9	50	—	—	—	—	9	ns
2013	Gallos[28]	Prospective	174	<40 to >60	ns	155	19	—	—	—	—	—	—	—	—	174	—	ns
2014	Gunderson[29]	Retrospective	46	24-63	18-70	—	17	—	29	41	—	12	—	5	—	14	20	1-84
2015	Orbo[30]	Retrospective	141	<44 to >52	<20 to >30	125	16	—	—	—	—	93	—	—	—	48	—	6
	Yang[31]	Retrospective	88	24-39	17-45	—	37	—	51	45	11	29	—	—	—	31[d]	—	5-14
	Zhang[32]	Prospective	27	ns	ns	—	19	—	8	—	—	27	—	—	—	—	—	—
2016	Tierney[33]	Retrospective	61	<40 to >40	<30 to >40	—	61	—	—	ns	—	ns	—	—	—	ns	—	1-29
	Reyes[34]	Retrospective	10	28-63	39-72	—	8	—	2	—	—	—	—	—	—	10	—	6-50
	Van Gent[35]	Retrospective	11	27-38	20-39	—	—	—	11	1	—	8	—	—	—	2[e]	—	6-19
	Wang[36]	Retrospective	21	ns	ns	—	—	15	6	—	—	21	—	—	—	—	—	ns
	Orbo[37]	Retrospective	141	<44 to >52	<20 to >30	125	16	—	—	—	—	93	—	—	—	48	—	6-24
	Li[38]	Retrospective	27	25-43	ns	—	19	—	8	—	—	27	—	—	—	—	—	2-8

(Continues)

TABLE 1 (Continued)

Year	Article	Study design	Sample size	Patients' features		Histology				Progestogen administered							Treatment duration (mo)
				Age	BMI	HWA	AH	EH	EEC	MGA	NETA	MPA	PG	OR	INJ	LNG	MIX	
2017	Fan[39]	Prospective	35	23-38	25-35	—	29	—	6	—	—	35	—	—	—	—	—	6-14
	Slettenn[40]	Retrospective	57 (43)[a]	30-70	27 (Mean)	—	—	57	—	—	—	31	—	—	—	26	—	3
	Zakhour[41]	Retrospective	84	24-55	19-64	—	57	—	27	—	—	—	—	43	3[f]	38[g]	—	3-90
Total	—	—	1360	19-79	17-72	629	422	140	204	159	31	634	2	48	3	503	29	1-90

HWA, endometrial hyperplasia without atypia; AH, atypical endometrial hyperplasia; EH, unspecified endometrial hyperplasia; EEC, early endometrial cancer; MGA, megestrol acetate; NETA, norethindrone acetate; MPA, medroxyprogesterone acetate; PG, progesterone; OR, unspecified oral progestogen; INJ, unspecified injectable progestogen; LNG, levonorgestrel-releasing intrauterine system; MIX, unspecified mixed progestogens; ns, not specified.

[a]Sample on which statistical association was assessed.

[b]5/36 patients received MPA and 6/32 received LNG for only 1 wk.

[c]1/16 patients also received NETA.

[d]14/31 patients also received NETA or MPA.

[e]2/2 patients also received MPA.

[f]3/3 patients also received OR.

[g]37/38 patients also received OR.

paired box gene 2 (PAX2),[27,40] cyclooxygenase-2 (COX2),[28] forkhead box protein O1 (FOXO1)[34] or β-catenin.[35]

With specific regard to post-treatment and changes assessment, 19 studies assessed 30 immunohistochemical markers on post-treatment biopsy and/or their changes during follow up.

PR, ER or their isoforms were assessed in seven studies. Both receptors showed a down-regulation in good responders and a stable expression in poor responders,[18,34] although five studies did not report any significant associations.[23,24,26,29,31]

Good responders showed increased expression of fas cell surface death receptor (Fas),[16] NCoR,[23] stromal Bcl2[24] and Dusp6,[32] and decreased expression of glandular Bcl2,[17,24] survivin,[22] Ki67,[23] Human epididymis protein 4 (HE4)[37] and sperm-associated antigen 9 (SPAG9).[38]

On the other hand, poor responders showed increased expression of GRP78,[33] nuclear factor erythroid 2-related factor 2 (Nrf2),[36,39] aldo-keto reductase family 1 member c1 (AKR1C1)[36] and surviving[39] and loss of PAX2,[30] loss of PTEN alone[30] and loss of PTEN combined with high phospho-mTOR.[21]

An increased Ki67 expression[31] and an increase in size of HE4-positive agglomerates[37] were the only two changes associated with relapse.

BAX,[17] p27,[21] SRC1,[23] p300/CPB,[23] SMRT,[23] caspase-3,[24] metallothionein (MT),[24] single-stranded DNA[26] and FOXO1[34] were not associated with outcome.

Details about the results are reported for each marker in Table 3.

4 | DISCUSSION

The search for predictive markers on pretreatment biopsy has the interesting aim to identify preventively the responders to the conservative treatment, avoiding the risk of disease progression linked to an ineffective therapy. In spite of the great number of markers assessed, significant associations were found for only few of them.

As expected, PR and ER were the most studied markers, since progestogens mediate their effects through PR, and an imbalance between progesterone and estrogens is involved in the pathogenesis of EH.[2] Although the results regarding PR and ER appeared variable, high expression of these receptors was predictive of good response in several studies.[25,28] In our previous study, we focused on ER and PR in the pretreatment phase. We found that they had significant predictive value only in women treated with LNG-IUS.[42] Such results might be due to the higher local action of progestogens provided by the intrauterine device. However, their predictive accuracy seemed to be insufficient for an actual clinical usefulness, although further studies are necessary to confirm these results. Moreover, it was impossible to analyze the predictive values of ER and PR isoforms. In the current study, PRB appeared to be the most promising isoform.[25,27]

Losses of PTEN and MMR have a recognized role in endometrial carcinogenesis.[2] As specifically discussed in our previous study, a

TABLE 2 Outcome considered, markers assessed and significant association found in the reviewed studies

		Outcomes considered					Statistically significant associations					
		Response		Relapse			Pretreatment			Follow-up		
Year	Article	Good	Poor	Yes	No	Immunohistochemical markers assessed	Marker	Outcome	*P*-value	Marker	Outcome	*P*-value
2003	Utsunomiya[15]	11	5	—	—	PR, ER, Ki67, 17β-HSD1, 17β-HSD2	High PR High 17β-HSD2	Good resp Good resp	<0.05 <0.01	ns	ns	ns
	Wang[16]	8	18	—	—	Fas, FasL	ns	ns	ns	high Fas ↑ Fas	Good resp Good resp	<0.05 <0.01
2005	Vereide[17]	43	14	—	—	Bcl2, BAX	None	None	None	↓ Bcl2 (glands)	Good resp	0.002
2006	Vereide[18]	15	14	—	—	PRA, PRB, ERα, ERβ, AR	None	None	None	Low PRA (stroma) ↓ PRA (glands) ↓ PRA (stroma) ↓ PRB (glands) ↓ PRB (stroma) ↓ ERα (stroma) ↓ ERβ (glands) ↓ ERβ (stroma)	Good resp Good resp Good resp Good resp Good resp Good resp Good resp Good resp	0.0079 0.0003 0.0186 0.0006 0.0012 0.0086 0.0186 0.0295
2007	Minaguchi[19]	21	8	9[a]	17[a]	PTEN, pAkt, p53, ER, PR	PTEN loss/ low pAKT	Poor resp	0.04	ns	ns	ns
	Yamazawa[20]	7	2	2[a]	5[a]	IGF1R, PTEN, ERα, PR, Ki67	High PR	Good resp	0.008	—	—	—
2008	Milam[21]	16	22	—	—	PTEN, p27, pmTOR	None	None	None	PTEN loss+high pmTOR	Poor resp	0.03
2009	Chen[22]	15	8	—	—	Survivin	ns	ns	ns	↓ survivin	Good resp	<0.001
	Kashima[23]	10	5	—	—	KI67, ER, PR, SRC1, p300/CBP, NCoR, SMRT	None	None	None	↑ NCoR ↓ Ki67	Good resp Good resp	<0.0077 <0.0076
2010	Orbo[24]	36	5	—	—	PRA, PRB, ERα, ERβ, Bcl2, caspase-3, MT	ns	ns	ns	Low Bcl2 (glands) high Bcl2 (stroma)	Good resp Good resp	0.03 0.01
	Akesson[25]	28	6	—	—	ERα, PRA, PRB, aromatase, PTEN	High ERα High PRA High PRB	Good resp Good resp Good resp	0.026 0.042 0.011	—	—	—
2011	Kamoi[26]	5	2	—	—	Ki67, ssDNA, ER, PR	None	None	None	None	None	None
2012	Upson[27]	81	33	—	—	PRA, PRB, PTEN, PAX2, Bcl2	High PRB	Good resp	0.011	—	—	—

(Continues)

TABLE 2 (Continued)

Year	Article	Outcomes considered: Response Good	Response Poor	Relapse Yes	Relapse No	Immunohistochemical markers assessed	Statistically significant associations: Pretreatment Marker	Pretreatment Outcome	Pretreatment *P*-value	Follow-up Marker	Follow-up Outcome	Follow-up *P*-value
2013	Gallos[28]	164	10	18	134	ER, PR, COX2, MLH1, Bcl2	High ER High PR	Good resp Good resp	0.001 0.02	—	—	—
2014	Gunderson[29]	30	16	7[a]	23[a]	ER, PR	None	None	None	None	None	None
2015	Orbo[30]	112	29	—	—	PAX2, PTEN	ns	ns	ns	PAX2 loss PTEN loss	Poor resp Poor resp	0.0003 0.0019
	Yang[31]	77	11	25	46	ER, PR, Ki67	None	None	None	High Ki67	Relapse	0.033
	Zhang[32]	21	6	—	—	Dusp6	High Dusp6	Good resp	<0.05	High Dusp6 ↑ Dusp6	Good resp Good resp	<0.05 <0.01
2016	Tierney[33]	25	36	—	—	GRP78	High GRP78	Poor resp	0.014	High GRP78	Poor resp	<0.001
	Reyes[34]	7	3	—	—	FOXO1, ER, PR, PRB	None	None	None	High ER High PRB ↓ ER ↓ PRB ↓ PR	Poor resp Poor resp Good resp Good resp Good resp Good resp	<0.05 <0.05 <0.01 <0.01 <0.05
	Van Gent[35]	6	5	5[a]	1[a]	PTEN, β-catenin	None	none	none	ns	ns	ns
	Wang[36]	11	10	—	—	Nrf2, AKR1C1	—	—	—	High Nrf2 high AKR1c1	Poor resp Poor resp	<0.0001 <0.0001
	Orbo[37]	123	18	50	73	HE4	ns	ns	ns	↓ HE4 ↑ HE4 granules' size	Good resp No relapse	<0.001 0.014
	Li[38]	21	6	—	—	SPAG9	ns	ns	ns	↓ SPAG9	Good resp	0.005
2017	Fan[39]	18	17	—	—	Nrf2, survivin	—	—	—	High Nrf2 High survivin	Poor resp Poor resp	<0.001 <0.001
	Slettenn[40]	43[a]	14[a]	10	33	ERα, ERβ, PRA, PRB, Bcl2, BAX, PTEN, PAX2	Low PRA (stroma) High PRB (glands)	Relapse Relapse	0.0044 0.0376	None	None	None
	Zakhour[41]	41	43	—	—	MLH1, MSH2, MSH6, PMS2	MMR loss	Poor resp	0.026	—	—	—

ns, not specified; none, no significant associations found; PR, progesterone receptor; PRA, progesterone receptor A; PRB, progesterone receptor B; ER, estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; AR, androgen receptor; PTEN, phosphatase and tensin homolog; p-AKT, phospho-protein kinase B; Bcl2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; PAX2, paired box gene 2; Nrf2, nuclear factor erythroid 2-related factor 2; MMR, mismatch repair proteins; Dusp6, dual-specificity phosphatase 6; GRP78, glucose-regulated protein-78; Fas, fas cell surface death receptor; FasL, Fas ligand; HE4, Human epididymis protein 4; SPAG9, sperm-associated antigen 9; AKR1c1, Aldo-keto reductase family 1 member c1; 17βHD2, 17β-hydroxysteroid dehydrogenase type 2; NCoR, Nuclear receptor co-repressor; p-mTOR, phospho-mammalian Target of rapamycin; IGF1R, insulin-like growth factor 1 receptor; SRC1, steroid receptor coactivator-1; p300/CBP, p300/CREB-binding protein; SMRT, silencing mediator for retinoid and thyroid-hormone receptors; MT, metallothionein; ssDNA, single-stranded DNA; COX2, cyclooxygenase-2; FOXO1, forkhead box protein O1; Ki67, antigen Ki67; p53, Tumor Protein p53; 17βHD1, 17β-hydroxysteroid dehydrogenase type 1.

[a]Excluded from statistical analysis.

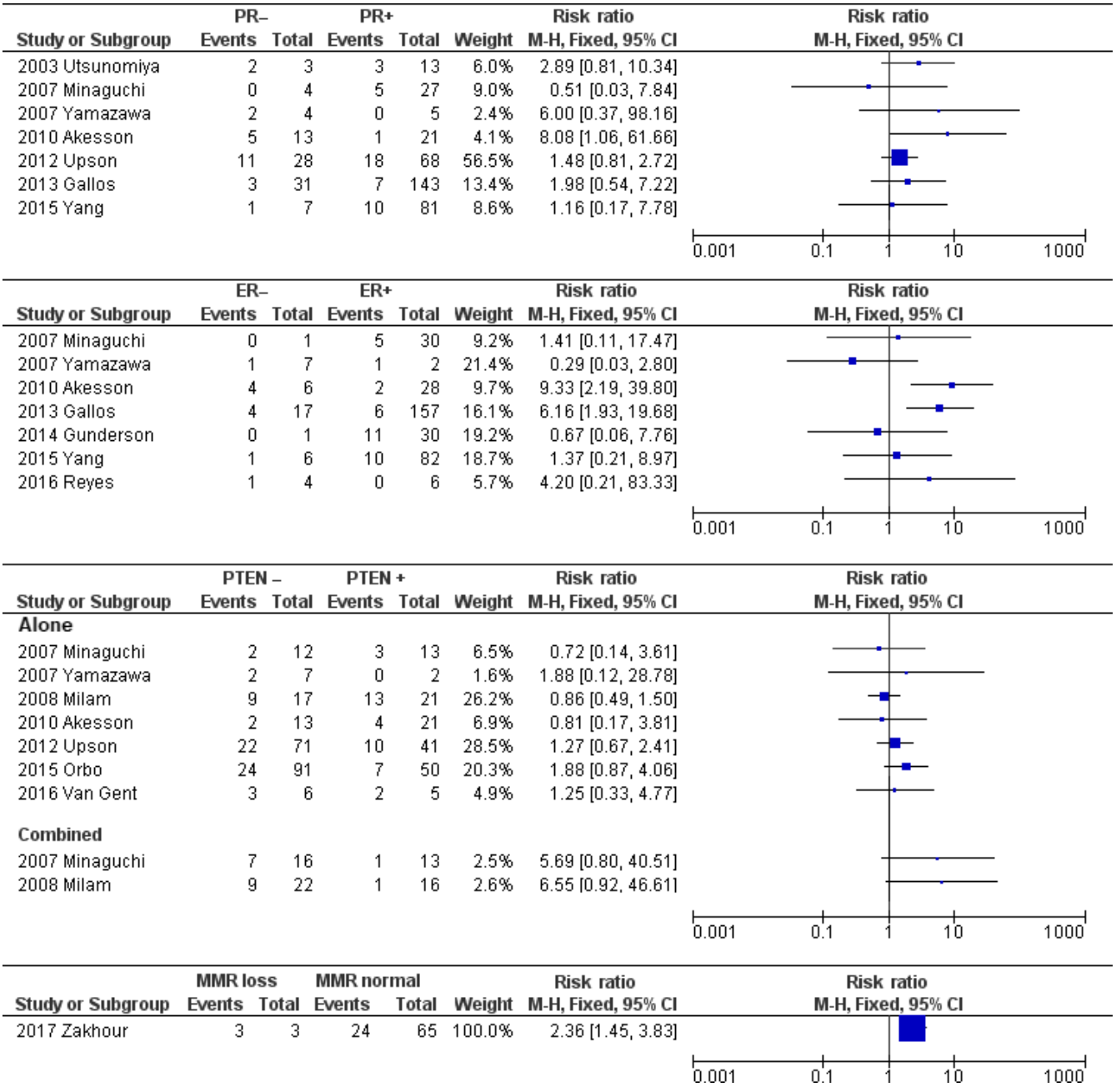

FIGURE 3 Relative risk of poor response for progesterone receptor (PR, pretreatment), estrogen receptor (ER, pretreatment), phosphatase and tensin homolog (PTEN, pretreatment) alone or combined with low phospho-AKT (2007 Minaguchi, pretreatment) or high phospho-mTOR (2008 Milam, follow up), and mismatch repair proteins (MMR, pretreatment) [Color figure can be viewed at wileyonlinelibrary.com]

loss of PTEN was never found to be significant alone.[43] It was predictive of poor response when combined with low expression of phospho-AKT, which is involved in the same pathway.[19] These results, in agreement with those regarding follow up and reported below, suggest that PTEN may have a predictive value when assessed in combination with other molecules of the same pathway. Regarding MMR, in a recent study, a loss of expression was associated with poor response in all cases.[41] This is a considerable result, though it is limited by the small number of patients with abnormal MMR pattern within the sample (only 6 of the 84 patients included showed loss of MMR).

Although Dusp6 enhances the growth-promoting effect of estrogens, its high expression was significantly predictive of good response.[32] High expression of GRP78, a key marker of endoplasmic reticulum stress, predicted poor response instead.[33] The enzyme 17β-HSD2, which catalyses the reversible interconversion of estrone and 17β-estradiol, predicted good response when highly expressed.[15] Despite the significant results found for Dusp6, GRP78 and 17β-HSD2, each of them was assessed in only one study (n = 27,[32] n = 61[33] and n = 16[15] respectively), thus, their usefulness needs to be confirmed by further studies.

TABLE 3 Results for each immunohistochemical markers

		Significant associations with the outcome				Nonsignificant	
		Pretreatment		Follow up			
Marker	N	High	Low/Absent	High/Increase	Low/Decrease	Pretreatment	Follow up
PR	9	Good response[15,20,28]	—	—	Good response[34]	Response[23,26,29,31,34]	Response[23,26,29,31]; Relapse[28,31]
PRA	5	Good response[25]	Relapse[40,a]	—	Good response[18]	Response[18,27]	Response[24]
PRB	6	Good response[25,27]; Relapse[40]	—	Poor response[34]	Good response[18,34]	Response[18]	Response[24]
ER	8	Good response[28]	—	Poor response[34]	Good response[34]	Response[15,23,26,29,31,34]	Response[23,26,29,31]; Relapse[28,31]
ERα	5	Good response[25]	—	—	Good response,[18a]	Response[18,20]; Relapse[40]	Response[24]
ERβ	3	—	—	—	Good response[18]	Response[18]; Relapse[40]	Response[24]
PTEN	8	—	Poor response[19b]	—	Poor response[21c,30]	Response[20,21,25,27,35]; Relapse[40]	—
Ki67	6	—	—	Relapse[31]	Good response[23]	Response[15,20,23,26,31]	Response[23]
Bcl2	5	—	—	Good response[24a]	Good response[17]	Response[17,27,28]; Relapse[40]	Relapse[28]
PAX2	3	—	—	—	Poor response[30]	Response[27]; Relapse[40]	—
BAX	2	—	—	—	—	Response[17]; Relapse[40]	Response[17]
Survivin	2	—	—	Poor response[39]	Good response[22]	—	—
Nrf2	2	—	—	Poor response[36,39]	—	—	—
MMR	1	—	Poor response[41]	—	—	—	—
MLH1	1	—	—	—	—	Response[28]	Relapse[28]
Fas	1	—	—	Good response[16]	—	—	—
p-AKT	1	—	Poor response[19b]	—	—	—	—
p-mTOR	1	—	—	Poor response[21c]	—	Response[21]	—
NCoR	1	—	—	Good response[23]	—	Response[23]	—
Dusp6	1	Good response[32]	—	Good response[32]	—	—	—
GRP78	1	Poor response[33]	—	Poor response[33]	—	—	—
HE4	1	—	—	No relapse[37d]	Good response[37]	—	—
AKR1C1	1	—	—	Poor response[36]	—	—	—
SPAG9	1	—	—	—	Good response[38]	—	—
17β-HSD2	1	Good respose[15]	—	—	—	—	—

(Continues)

TABLE 3 (Continued)

		Significant associations with the outcome				Nonsignificant	
		Pretreatment		Follow up			
Marker	N	High	Low/Absent	High/Increase	Low/Decrease	Pretreatment	Follow up
FasL	1	—	—	—	—	—	Response[16]
AR	1	—	—	—	—	Response[18]	Response[18]
p53	1	—	—	—	—	Response[19]	—
IGF1R	1	—	—	—	—	Response[20]	—
p27	1	—	—	—	—	Response[21]	Response[21]
SRC1	1	—	—	—	—	Response[23]	Response[23]
p300/CBP	1	—	—	—	—	Response[23]	Response[23]
SMRT	1	—	—	—	—	Response[23]	Response[23]
caspase—3	1	—	—	—	—	—	Response[24]
MT	1	—	—	—	—	—	Response[24]
aromatase	1	—	—	—	—	Response[25]	—
ssDNA	1	—	—	—	—	Response[26]	Response[26]
COX2	1	—	—	—	—	Response[28]	Relapse[28]
FOXO1	1	—	—	—	—	Response[34]	Response[34]
B-catenin	1	—	—	—	—	Response[35]	Response[35]
17β-HSD1	1	—	—	—	—	Response[15]	—

N, number of studies assessing the marker.
[a]Only stromal expression.
[b]Combined variable "PTEN loss or low p-AKT".
[c]Combined variable "PTEN loss + high p-mTOR".
[d]Increased size of HE4-positive granules was associated with absence of relapse.

Overall, given the possibility that several mechanisms may support the resistance to progestogens, we think that it may be more appropriate to search for a predictive immunohistochemical panel rather than a single predictive marker.

On the other hand, the assessment of post-treatment markers and their changes during follow up have the aim to evaluate the efficacy of therapy and to investigate the mechanisms of action and resistance. In this regard, the prediction of the individual response in an early phase of therapy may allow the timing of follow up to be adapted and, if necessary, changing the treatment.

The significant markers found in pretreatment assessment still appeared relevant on follow up. In fact, in some studies PR and ER showed a down-regulation in good responders[18,34]; loss of PTEN combined with high expression of phospho-mTOR (both involved in the same pathway) appeared predictive of poor response[21]; high and increasing expressions of Dusp6 and GRP78 were still associated with good and poor response, respectively.[32,33] Regarding MMR, since their loss characterized hyperplasias that persisted on follow up, they still may be significant in this phase, although any changes in them were not assessed.[41]

Among the markers of good response, Fas and nuclear receptor co-repressor (NCoR) appeared to contribute to the growth suppression mediated by progestogens.[10,23]

Among the markers of poor response, survivin, Nrf2 and AKR1C1 appeared involved in the same pathway supporting the resistance to progestogens,[22,36,39] whereas HE4 and SPAG9 were found to promote tumor growth.[37,38] They were proposed as potential targets for new therapies in progestogen-resistant cases.

A decrease of the proliferation marker Ki67[23,31] and of the anti-apoptotic protein Bcl-2[17,24] appeared to be consequent on a successful treatment, consistently with the pro-apoptotic effect of progesterone. Similarly, good responders showed a disappearance of glands negative for the oncogenic protein PAX2.[30]

Although the markers of good response may provide new elements on the mechanism of action of progestogens, their potential role as predictive markers may be limited by the fact they were detected in most cases in normal endometrial glands, after a total regression of disease. Thus, they may not be informative about the responsiveness of pathologic glands. On the other hand, the markers of poor response were assessed in persistent EH and EEC, and their results may therefore be more relevant in this field.

While assessing these results, it should be considered that they actually consider two different pathologic conditions. In fact, most EH without atypia are benign proliferations due to an unopposed action of estrogens, whereas atypical EH and EEC are neoplastic lesions characterized by specific underlying mutations.[1,44] It might be expected that progestogens are more effective in functional condition such as benign EH, rather than in premalignant EH or EEC. Mechanisms of resistance may differ in these two conditions, as well as the association of immunohistochemical markers with the response. Some markers are typically altered in premalignant EH/EEC but not in benign EH.[45-48] In our review, most studies assessed EH without atypia vs atypical EH and/or EEC (Table 1), which may create a bias in the results. Therefore, results about PR, 17β-HSD2, MMR, PTEN, Dusp6, GRP78, Nrf2 and survivin may be more significant, since they were assessed in populations constituted exclusively of neoplastic lesions (atypical EH and EEC).

Our review assessed the role of immunohistochemical markers in predicting the outcome of the conservative therapy of EH and EEC. To the best of our knowledge, no similar review or meta-analysis on the topic is present in the literature.

Our study tried to provide a complete, clear and accurate overview of all the available results in this field. Our aim was to help future researchers in directing their efforts towards the more promising markers.

On the other hand, the inhomogeneity displayed by the reviewed studies also represents an important limitation of our results.

First of all, the sample size was exiguous in several studies, in two cases not even reaching 10.[20,26] The study populations included patients highly variable as to age and body mass index (Table 1). This may be a confounding factor, since post-menopause and obesity were proposed as factors negatively influencing the therapy response.[49,50] Nevertheless, in a meta-analysis published on 2014, they did not significantly influence the outcome.[12]

In 11 of the 26 reviewed studies, the sample included EH without atypia together with atypical EH and/or EEC. Moreover, in three articles the type of EH is not specified (Table 1). As discussed above, atypical EH and EEC should be considered separately from EH without atypia, given the benign nature of the latter.

In most studies, different progestogens were administered with variable treatment duration (Table 1). This may represent important confounding factors, since regression rates vary among different treatments. LNG-IUS appears to be the most effective treatment[9,51,52] and it seems to perform even better if combined with hysteroscopic resection.[53] The treatment duration and the recommended follow up for outcome evaluation should be at least of 6 months.[6]

The different histological definition of response and the different methods used to grade the expression of the markers may be other important confounding factors.

Given these observations, we consider that further studies on this field may achieve more significant results if they include only patients treated with LNG-IUS and consider EH without atypia (benign) and atypical EH (premalignant) separately, searching for a prognostic immunohistochemical panel rather than a single significant marker.

5 | CONCLUSION

PR and ER were the most studied predictive markers in both pretreatment and follow-up phase, showing conflicting results. The study of PR and ER isoforms may lead to better results; PRB appeared as the most promising. MMR, Dusp6, GRP78 and PTEN combined with phospho-AKT or phospho-mTOR showed significant results but were evaluated in only one study each; thus, further studies are needed to define their accuracy.

Nrf2 and survivin were the most significant markers in premalignant EH and in the follow-up phase. Despite being unspecific, Bcl2 and Ki67 may also reasonably play a role. Further studies are necessary for Fas, NCoR, AKR1C1, HE4, PAX2 and SPAG9, since they were only assessed in one study each.

Hopefully, a predictive panel of immunohistochemical markers will be elaborated in the future.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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